Structure and Development of the Asbestos Body

Y. Suzuki, M.D., and J. Churg, M.D.

MARCHAND,[1] in 1906, was the first to report bizarre and distinctive golden-brown bodies in human lung tissue. Others later correlated these odd structures with exposure of the patient to asbestos;[2-5] hence the designation "asbestos bodies." Over the years many have studied their architecture and composition. It now is generally accepted that they consist of asbestos fibers coated by protein and iron, and probably other material.

Asbestos bodies can be produced experimentally in a number of animal species including the guinea pig, white mouse, hamster, monkey, and rabbit; their development can be followed after inhalation or instillation by the animal of asbestos into the lungs.[6-12]

It is generally thought that formation of asbestos bodies is a biologic process, the result of interaction between the asbestos fibers and the alveolar macrophages. The details of this process are still obscure: the mechanism of the phagocytosis of asbestos; cell types which have the potential for such phagocytosis and asbestos body formation; and the interrelationships among phagocytosis, intracellular formation of the coating of the body, and development of the body. Davis [12] believes that asbestos body formation is essentially an intracellular process and that it is initiated by deposition of ferritin molecules around the phagocytosed asbestos fibers. He also suggested that some of the macrophages containing asbestos eventually transform into fibroblasts, thus forming a link between the intracellular presence of asbestos fibers and the collagenous fibrosis which is a characteristic feature of pulmonary asbestosis.

While this mechanism may be of importance, it is by no means certain whether it is the only or even the most common way by which pulmonary fibrosis develops. Other alveolar cells, such as alveolar epithelial cells and septal cells, also participate in reactions to asbestos. It is known that the septal cell, which is probably the only cell of mesen-

---

From the Environmental Science Laboratory, Department of Community Medicine, and the Department of Pathology, Mount Sinai School of Medicine of the City University of New York, New York, N. Y.

Supported by U. S. Public Health Service Research Grants AM-00918 and UI-00496.

Accepted for publication Jan. 7, 1969.

Address for reprint requests: Dr. Churg, Department of Pathology, The Mount Sinai Hospital, 100th St. and Fifth Ave., New York, N. Y. 10029.

chymal origin in the alveolar wall, is intimately concerned with formation of collagen and elastin under normal conditions. It is also uncertain whether ferritin is the main constituent of the coating of asbestos bodies. Although Davis did not observe any alterations of cell organelles in the alveolar macrophages during the development of the asbestos body, it seems possible that cell organelles are intimately related to the process of asbestos body formation.

It was decided, therefore, to restudy the formation of asbestos bodies on the ultrastructure level, using the hamster as the experimental animal and chrysotile asbestos as the mineral. The hamster has considerable resistance to infection, which often occurs in the rat following intratracheal instillation of asbestos. Chrysotile asbestos has a rather constant diameter (150–450 Å) and a very characteristic tubular structure of its unit fiber, readily recognizable under the electron microscope.[13] Thus, chrysotile is not only a specific marker, but may also serve to determine the effect of biologic environment upon the structure of the fiber.

Materials and Methods

A total of 24 male hamsters, ranging from 50 to 130 gm. in weight, were given 1 mg. of soft* chrysotile asbestos suspended in 0.1 ml. of normal saline. This was administrated intratracheally under diabutyl anesthesia (0.6 ml. intraperitoneally).†

The chrysotile fibers used in this study averaged 6 μ in length. This did not take into account fibers of submicroscopic dimensions. The appearance and size of fibers were checked under the light and the electron microscopes. For the latter purpose the suspension of asbestos was diluted in double-distilled water approximately 50 times; one drop of the diluted suspension was sprayed on the surface of formvar membrane and allowed to dry. The animals in the first group were sacrificed successively at 1, 2, 3, 5, 7, 12, and 16 days after instillation of asbestos, and those of the second group were killed 6, 12, and 24 months after instillation. Three additional hamsters were used as controls. One of these was given 0.1 ml. of normal saline intratracheally to check the effect of normal saline, and the other two served as untouched controls.

For electron microscopy, three different methods of fixation were used: (1) Under diabutyl anesthesia, 20 ml. of Ringer solution was infused into the animals through the inferior vena cava and drained from the common iliac artery. Ringer solution was followed by perfusion of phosphate-buffered 2% glutaraldehyde at

* The terms "soft" and "harsh" used to describe the fiber types reflect readily measurable differences in "feel" to touch. These terms originated in the commercial literature in which fiber "softness" reflected its ability to be woven into valuable products—i.e., its relative flexibility. Harsh chrysotile fibers have also been described as "splintery" in nature. Harsh-soft and splintery-flexible properties reflect basic differences in both chemical and physical makeup of the fiber types, which account for the observably different physical behavior patterns. Differences in structural water content, bulk oxide composition, trace elements, amount of inclusions, thickness of amorphous coating, and structural ordering have been suggested as causative factors in these differences.[14]

† Intratracheal instillation was performed under the supervision of Dr. William E. Smith.

pH 7.2 for 10 min. After that, the chest was opened and small pieces of lung were selected and cut into cubes less than 1 cu. mm. in diameter. After additional fixation for 1 hr. at 4° C. in the same glutaraldehyde solution, the tissues were transferred into 1% osmium tetroxide in phosphate buffer for postfixation. (2) The procedure was the same as in (1) except that 2.5% potassium bichromate was used as an intermediate step between glutaraldehyde and osmium fixation, as described by Sugihara *et al.* (triple fixation).[15] (3) Under anesthesia, the chest was opened without prior perfusion, and the cubes of tissue under 1 cu. mm. in diameter were immersed directly in 1% phosphate-buffered osmium tetroxide at 4° C. for 2 hr. After fixation, the specimens were dehydrated through graded ethanol and embedded in epoxy resin. Sections were stained with uranyl acetate followed by lead, or with lead only. Electron micrographs were taken with an RCA 3G or a Hitachi 11-B microscope at 100 kv.

For phase microscopy, 1-μ sections were cut from all blocks in order to select suitable areas for electron microscopy.

For light microscopy, the tissues were fixed in 10% neutral formalin. After paraffin embedding, 4-μ sections were stained with hematoxylin and eosin (H&E), Mallory trichrome, Prussian blue, periodic acid-Schiff reagents (PAS), Van Gieson, and Gomori silver reticulum stain.

Observations

Light Microscopy

Early changes in the lung 1–3 days after exposure to asbestos were focal and consisted of (1) severe acute inflammation centering upon bronchioles but extending also into the alveoli (Fig. 1); (2) exudation of edema fluid in alveolar spaces with or without formation of giant cells (Fig. 1); and (3) diapedesis of erythrocytes into the alveolar spaces and erythrophagocytosis by alveolar macrophages. Surprisingly, the asbestos fibers could not be demonstrated under light microscopy, including phase and polarized microscopy.

At 7–12 days after exposure, exudative changes, edema, and leukocyte infiltration decreased. However, focal thickening of the alveolar wall and swelling of the alveolar lining became evident. Peribronchiolar inflammation was still considerable, and there were numerous mononuclear macrophages, which gave weakly positive reactions with Prussian blue staining. The asbestos fibers were still undemonstrable.

At 16 days after exposure, the macrophages gave strong reactions with Prussian blue staining. The cytoplasm of the macrophages was diffusely blue, though some of the cells gave only focal reactions showing only tiny blue granules or needle-like structures in their cytoplasm. Some of these needles resembled small asbestos bodies in shape. Hypercellularity and slight thickening of alveolar wall were seen at this time (Fig. 2).

After 6 months, typical asbestos bodies, brown in color in H&E stain and blue with the Prussian blue technique, were seen in the macrophages. At this time, they were usually less than 10 μ in length and rod-

shaped; the cytoplasm surrounding the bodies occasionally stained faintly with iron staining. In addition to the appearance of many typical asbestos bodies, the proliferative alteration, hypercellularity of the alveolar wall, plasma cell infiltration in the stroma, and reticular fibrosis were prominent.

In the material seen 1 and 2 years after exposure, variously shaped asbestos bodies, comma-like and segmented, were observed (Fig. 3 and 4). They gave strong reactions with Prussian blue (Fig. 4), and were usually shorter than 15 μ, but, rarely, structures over 50 μ in length were observed. It was noteworthy that even in this stage some of the asbestos bodies were still surrounded by Prussian blue-positive cytoplasm of macrophages.

In these late stages, the proliferative alteration was more advanced, extra- and subpleural fibrosis was seen in some lobes, and PAS staining revealed thickening of the alveolar basement membranes. Around small bronchi or bronchioles, adenomatous proliferation of epithelial cells was seen. There were also foci of mild hemorrhage.

Electron Microscopy

Ultrastructure of Chrysotile Fibers

The fibers used for intratracheal instillation varied in length and thickness, as seen in Fig. 5. Under high magnification they consisted of bundles of "unit fibers." The latter were fairly constant in width, ranging from 270 to 300 Å and had a typical tubular structure with a central "capillary" space (Fig. 6). The structure of the unit fiber could be recognized easily in sections and was not significantly altered by fixation, embedding, and sectioning (Fig. 7). Unit fibers cut longitudinally showed the same bridged tubular arrangement (Fig. 7); however, some appeared altered, as discussed later.

Ultrastructure of Asbestos Bodies

The bodies were located usually within the cytoplasm of a variety of cells, including alveolar macrophages, stromal cells of the septums, and even neutrophilic and eosinophilic polymorphonuclear leukocytes (Fig. 8–12, 14, and 16). Some bodies were extracellular (Fig. 13). Although they varied in size and shape, all bodies had the same basic structure —a core of tightly or loosely packed asbestos unit fibers surrounded by a coat of iron micelles (Fig. 8–12) and separated from the cell cytoplasm by a limiting membrane; this membrane is similar to those of phagosomes or lysosomes. The extracellular bodies lacked the limiting membrane (Fig. 13). Such "naked" bodies, as well as free asbestos

fibers, were present in the alveoli not only early in the experiment but frequently also in the late stages. The significance of this finding will be discussed later.

As a rule the bodies rarely exceeded 20 μ in length, though in animals sacrificed at 24 months there were very large structures, 50–60 μ long. Many bodies were under 1 μ and could be recognized only with the electron microscope (Fig. 12).

The tubular structure of the unit fibers in the core of the asbestos bodies was frequently, though not always, preserved (Fig. 9, inset). The iron micelles coating the fibers showed a subunit structure similar to that of ferric hydroxide of the ferritin molecule (Fig. 10, inset). In some bodies, fine fibrils 30 Å in diameter could be seen in concentric arrangement (Fig. 11, inset). The space enclosed by the limiting membrane tended to conform to that of the enclosed asbestos body. Often the membrane was separated from the layer of iron particles by a zone of amorphous material (Fig. 8–11).

Morphogenesis of Asbestos Bodies

The early stages of asbestos body formation occur on a subcellular level and can be followed only with the electron microscope. Moreover, the light microscopic picture is obscured by the rapid exudation of inflammatory cells, mainly alveolar macrophages and polymorphonuclear leukocytes.

Although the instilled asbestos fibers average 6 μ in length and about 1 μ in diameter, the majority of fibers seen in the alveoli are less than 1 μ long and are often only the thickness of a unit fiber. Therefore, as shown in Fig. 17, the identification of these submicroscopic fibers was extremely difficult even in an electron micrograph of low magnification. This suggests that the instilled fibers tend to split longitudinally and to break up into short fragments. As a rule, formation of asbestos bodies begins with phagocytosis of asbestos fibers by a variety of cells (see *Discussion*): alveolar macrophages (both free and fixed), alveolar epithelial cells, stromal cells of the alveolar wall (probably derived from alveolar septal cells), and polymorphonuclear leukocytes.

The unit fibers of chrysotile, as well as naked asbestos bodies, devoid of limiting membranes, were observed frequently in the alveolar spaces even in the late stages (Fig. 7). It is probable that these structures were present in macrophages at one time and became liberated upon the death of the cell. The fibers are probably rephagocytosed, as suggested by the presence of early stages of asbestos bodies even 24 months after exposure.

The process of phagocytosis is quite similar to that of microphagocytosis or micropinocytosis of other types of particles. We have observed the successive steps of phagocytosis by alveolar macrophages: formation of pseudopods, direct contact of fibers with the plasma membrane, formation of a recess containing a fiber with pinching off from the plasma membrane to form a phagosomal vacuole, and the movement of the vacuole into the deeper zones of cytoplasm (Fig. 18–24). The phagosomes were not uniform in shape and content. They were not always round, but very frequently elongated or rod-shaped, with the long axis usually corresponding to that of phagocytosed fibers. Their contents varied from being electron-opaque to electron-lucent. Some of them contained, in addition to fibers, osmiophilic lamellar structures, and others were filled by homogeneous osmiophilic substance (Fig. 20 and 22–24). Some of these vacuoles showed the structure of the digestive vacuole or residual body described by de Duve and Wattiaux,[16] strongly suggesting that at least some of these vacuoles are lysosomes, though their enzymatic activity was not examined in this study (Fig. 20 and 22–24). It was interesting to note that the unit fibers within phagosomal vacuoles usually remained parallel even when separated from each other (Fig. 20–25 and 27). Tonofilaments of the macrophage were sometimes found around the limiting membrane of the phagosome, as if these filaments supported the phagosomal vacuoles in the cytoplasm (Fig 21). During the initial steps of phagocytosis, no iron micelles were seen in the environment of phagocytic cells, and no micelles were present in their cytoplasm during the process of phagocytosis and formation of phagosomes.

The next step in the formation of the asbestos body was associated with the appearance of iron micelles in the cytoplasm of cells that have the ingested asbestos fibers. In our material this was seen 16 days after intratracheal instillation. The micelles were packed into granular aggregates, round or occasionally elongated or rod-shaped, from 0.2 to 0.5 μ (average 0.3 μ) in diameter. These aggregates were scattered more or less uniformly throughout the cytoplasm. Each aggregate was surrounded by a limiting membrane and gave a positive reaction for iron by the Prussian blue method. With higher magnification, some of the iron micelles exhibited the structural characteristics of ferritin molecules (Fig. 10, inset). These features identify the aggregates as hemosiderin granules.[17–20]

In addition to the typical hemosiderin granules, there were smaller structures, 0.1–0.4 μ in diameter, consisting of densely packed iron micelles, but devoid of a limiting membrane (Fig. 26, inset). These

smaller granules often were related closely to the well developed cell organelles—rough-surfaced endoplasmic reticulum, free ribosomes, and especially the mitochondria in the peripheral region of the cytoplasm (Fig. 25–27). These mitochondria were also in close contact with the typical membrane-invested hemosiderin granules. Richter [19,20] has proposed that the latter develop from mitochondria via an intermediate form which he called "siderosome." However, we have not observed such transformation in our material. "Coating vesicles" frequently were seen around the hemosiderin granules. Granules located in the Golgi zone were of less compact structure, their iron micelles being distributed in a homogeneous matrix (Fig. 27).

Simultaneously with their appearance in the cytoplasm, iron micelles also became visible in the phagosomes which contained asbestos fibers (Fig. 25 and 27). The micelles were comparatively few in number, arranged around the fiber, and embedded in a pale homogeneous matrix (Fig. 25 and 27). Such structures could not be recognized as asbestos bodies by light microscopic examination, but by electron microscopy they bore an unmistakable resemblance to the typical iron-rich asbestos bodies. These early structures may be called "immature" asbestos bodies. By continuous accretion of iron micelles, they were converted into "mature" bodies.

The lung tissue of animals sacrificed at 6–24 months contained a large number of mature bodies, but also many uncoated fibers and immature bodies. As shown in Fig. 10, even in this late stage, "caveola intracellulare"-like structures were present on the limiting membrane of mature asbestos bodies. Sometimes, the limiting membrane was covered focally by "fuzzy" material (Fig. 8). The surrounding cytoplasm contained well developed hemosiderin granules and also small vesicles which resembled Golgi vesicles in size and shape and contained iron micelles (Fig. 12). The structure of the iron-containing coat of the asbestos body was quite similar to that of a hemosiderin granule, except that in the granules the iron micelles were distributed evenly in the ground substance, while in the coat the micelles were concentrated close to the fiber and the periphery near the limiting membrane was formed mainly by the ground substance.

Discussion

Structure and Formation of Asbestos Body

The two main components of the asbestos body—i.e., a central fiber of asbestos and a peripheral coat of iron—have been known since the work of Cooke,[2] Stewart and Haddow,[3,8] Lynch and Smith,[5] and

Gloyne.[22] To these components we would like to add a third element essential for the development of the body—the "limiting membrane," which invests and separates asbestos from the surrounding cell cytoplasm. Formation of the asbestos body must be considered an active biologic process, the result of interaction of asbestos fibers with living cells. The first step in this process is phagocytosis of fibers by various cells (especially macrophages) in the lung (and perhaps also in other organs and tissues). The process of phagocytosis of asbestos is similar to that of other substances (e.g., India ink and Thorotrast) by alveolar macrophages.[23,24] This process has been termed "en-membranosis" by Tanaka.[25] It must be emphasized that in our material the process of phagocytosis and of subsequent segregation of asbestos in intracytoplasmic vacuoles could be seen only with the electron microscope. The main reason for this is probably extensive splitting and fragmentation of chrysotile asbestos in the lung.

The vacuoles containing asbestos were similar in structure to digestive vacuoles, phagosomes, or lysosomes. The purpose of this segregation undoubtedly is to isolate the potentially or actively injurious material from the cytoplasm and to create conditions for its digestion, elimination, or neutralization.

The cell apparently does make attempts at digestion of asbestos. This is evidenced by the altered appearance of some fibers in the vacuoles with loss of electron density and decrease in thickness of the capillary wall (Fig. 28). The presence of autophagic vacuoles or residual bodies strongly suggests that phagosomes containing asbestos are transformed into secondary lysosomes.[16] The process of coating of the asbestos fiber by iron is much more common than digestion and possibly represents an attempt at neutralization of their injurious properties. Iron micelles are seen in the vacuoles around the asbestos fibers quite early—in our material 16 days after instillation of abestos. Simultaneously, iron also appears in the cell cytoplasm in the form of hemosiderin granules. The presence of caveolae intracellulares, as well as fuzzy material on the limiting membrane of the vacuoles, suggests transfer of iron between the cytoplasm and the vacuoles by fusion of iron-bearing vesicles with the limiting membranes of the vacuoles.

Formation of asbestos bodies is a continuous process. Hemosiderin granules are present in the cytoplasm of cells containing not only early but also mature bodies. The latter grow in thickness and perhaps also in length by continuous addition of iron. The stimulus that initiates and maintains this accretion of iron is not actually known. Undoubtedly, it resides in the asbestos fiber, but whether it is chemical or physical in nature has not been determined.

The source of iron must be looked for in the tissue of the organism that contains the asbestos.[2,4] The opposite point of view that iron is leached out of asbestos fibers and deposited on their surface by the action of tissues [26] is hardly tenable. Asbestos bodies are formed readily by iron-poor as well as iron-rich asbestos; often the amount of iron in the coating far exceeds the amount initially present in the fiber.

Both the iron in the cytoplasm of the cells containing asbestos and the coat of asbestos bodies have the structure of hemosiderin. Davis [12] has suggested that the coat consists of ferritin. While ferritin undoubtedly is present, there are also many smaller micelles, under 50 Å in diameter, whose structure differs from that of ferritin.[18] The homogeneous ground substance in which the various iron micelles are embedded is a characteristic feature of hemosiderin.[18] It is undoubtedly also the important source of protein in the coat of asbestos bodies.

According to current concepts, hemosiderin contains ferric hydroxide in various forms.[18-20,27] Several types of hemosiderin are recognized, some are very similar to the iron granules in the cytoplasm of asbestos containing phagocytes, except that we have not observed ferritin crystals.

Hemosiderin probably is formed locally in the phagocytic cells. The necessary iron may come from several possible sources, two of which seem most likely. The first is the breakdown of hemoglobin from phagocytosed or hemolyzed red blood cells. We did observe erythrophagocytosis in our material (Fig. 17), though not very often and mainly during the early stages of asbestosis. It is also known that certain types of asbestos, especially chrysotile, are capable of inducing hemolysis in vitro.[28] The relation of this hemolysis to formation of asbestos bodies in vivo is uncertain, but deserves further investigation. The second likely source is the iron stored in the lungs and elsewhere in the body, and the transferrin-bound iron in the circulating plasma. The relative importance of these and other sources is a matter for further study.

Of interest is the presence of uncoated asbestos fibers and immature bodies in the late stages of the experimental disease. It is possible that some of the fibers were never phagocytosed and never coated with iron. More likely, however, is the possibility that many of the uncoated fibers exert a toxic effect upon their host cell, and interfere with its normal function. Eventually upon the death of the cell, they are released in the form of uncoated fibers or lightly coated immature bodies. Maturation of bodies may involve repeated phagocytosis and release. Whether the opposite process, dissolution of the iron coat, occurs in the lung is presently unknown. It seems likely that many asbestos fibers are phago-

cytosed and released many times without being converted into asbestos bodies.

Cells That Produce Asbestos Bodies

The ability of the alveolar macrophages to phagocytose asbestos fibers is well known. In addition, we have observed phagocytosis by alveolar epithelial cells (both Types A and B), by cells in the septal stroma, and by leukocytes. Of these, the epithelial cells and the stromal cells potentially are capable of forming asbestos bodies, because they contain or form hemosiderin granules. There appears to be close relationship between the epithelial cells and the alveolar macrophages, the latter apparently being derived, at least to some degree, from the former.[29] Karrer[23] has suggested that the phagocytic cells in the alveolar stroma represent alveolar macrophages which have migrated into the wall. This is not a very likely source of phagocytic stromal cells in our animals, whose alveolar basement membranes were very thick (up to 10 times the normal thickness) in the late stage of this experiment and consequently likely to impede migration of cells. We would like to suggest that the stromal phagocytes originate from the alveolar septal cells.

Although neutrophilic and eosinophilic polymorphonuclear cells on occasion contain asbestos fibers and asbestos bodies (Fig. 14–16), they do not form hemosiderin granules. It seems safe to say that leukocytes do not produce asbestos bodies, but only phagocytose those that have been released from degenerating cells of other types.

Role of Submicroscopic Asbestos Fibers

There has been considerable debate in the literature on the importance of fiber length in the induction of acute and chronic tissue response to asbestos.[6,11,30] This response includes polymorphonuclear exudation, mononuclear cell proliferation, formation of giant cells, fibrosis of the pulmonary parenchyma and pleura, and adenomatous proliferation of the terminal bronchiolar epithelium. Both shorter fibers (under 5 μ) and longer fibers (over 20 μ) have had their advocates. With the application of electron microscopy, it became clear that measurement of the average fiber length in suspension is highly unreliable, because no account is taken of submicroscopic fibers under 1 μ long. We found very large numbers of such fibers in the lungs of our animals. Some of them were present in the original suspension, but the majority seem to be derived from longer fibers which became fragmented and split. The submicroscopic fibers are not only very numerous, but are also of the right size to be ingested by, and to react with, individual cells of the

lung. We suggest that it is the submicroscopic fibers that are responsible for most of the biologic effects of asbestos.

Summary

Formation of asbestos bodies in the hamster lung from instilled chrysotile asbestos was followed by means of light, phase, and electron microscopy. This process took place in the cytoplasm of certain pulmonary cells, especially the alveolar macrophages, and, to a lesser extent, alveolar epithelial cells and septal cells. The successive steps consisted of: (1) phagocytosis of small fragments (often under 1 μ long and 300 Å in diameter) and incorporation in cytoplasmic phagosomes; (2) appearance of hemosiderin in the cytoplasm of the cells; (3) intracellular transportation of iron micelles from hemosiderin granules into the phagosomes; and (4) progressive concentration of the iron micelles in the vicinity of the fiber and partial clearing of the protein ground substance along the periphery of the phagosome. The central fiber, the coat of hemosiderin, and the investing membrane of the phagosome are considered to be the essential elements of an asbestos body.

The presence of uncoated asbestos fibers is noted even after the lapse of 2 years. It is suggested that such fibers, in fact, have undergone phagocytosis, but have been released due to the death of the cell before they became coated with iron. These uncoated fibers probably continue to interact with the cells and may be responsible for the progressive alteration of the lung tissue.

References

1. Marchand, F. Über eigentümliche Pigmentkristalle in den Lungen. *Verh Deutsch Ges* 17:223–228, 1906.
2. Cooke, W. E. Asbestos dust and the curious bodies found in pulmonary asbestosis. *Brit Med J* 2:578–580, 1929.
3. Stewart, M. J., and Haddow, A. C. Demonstration of the peculiar bodies of pulmonary asbestosis (asbestosis bodies) in material obtained by lung puncture and in the sputum. *J Path Bact* 32:172, 1929.
4. Simson, F. W., and Strachan, A. S. Asbestosis bodies in the sputum: A study of specimens from 50 workers in asbestos mill. *J Path Bact* 34:1–4, 1931.
5. Lynch, K. M., and Smith, W. A. Asbestosis bodies in sputum and lung. *JAMA* 95:659–661, 1930.
6. Vorwald, A. J., Durkan, T. M., and Pratt, P. C. Experimental studies of asbestosis. *A.M.A. Arch Industr Hyg* 3:1–43, 1951.
7. Gardner, L. U., and Cummings, D. E. Studies on experimental pneumonokoniosis: inhalation of asbestos dust; its effect upon primary tuberculous infection. *J Industr Hyg* 13:65–81, 97, 1931.
8. Stewart, M. J. Asbestosis bodies in the lungs of guinea-pigs after three to five months exposure in an asbestos factory. *J Path Bact* 33:848, 1930.

9. WAGNER, J. C. Asbestosis in experimental animals. *Brit J Industr Med 20*: 1–12, 1963.

10. GROSS, P., and DE TREVILLE, R. T. P. Experimental asbestosis: Studies on the progressiveness of the pulmonary fibrosis caused by chrysotile dust. *Arch Environ Health (Chicago) 15*:638–649, 1967.

11. HOLT, P. F., MILLS, J., and YOUNG, D. K. The early effects of chrysotile asbestos dust on the rat lung. *J Path Bact 87*:15–23, 1964.

12. DAVIS, J. M. Electron microscope studies of asbestosis in man and animals. *Ann NY Acad Sci 132*:98–111, 1965.

13. GAZE, R. The physical and molecular structure of asbestos. *Ann NY Acad Sci 132*:23–30, 1965.

14. LANGER, A. M., and KERR, P. F. The nature of harsh and soft chrysotile. Paper No. 2-2, Oxford Conference on Asbestos Minerals, 1967.

15. SUGIHARA, R., LEE, K. J., SUGIOKA, T., and YASUZUMI, G. "Experiments on triple fixation for electron microscopy. *Electron Microscopy (Tokyo) 2*:25, 1966.

16. DE DUVE, C., and WATTIAUX, R. Functions of lysosomes. *Ann Rev Physiol 28*:435–492, 1966.

17. RICHTER, G. W. Electron microscopy of hemosiderin; presence of ferritin and occurrence of crystalline lattices in hemosiderin deposits. *J Biophys Biochem Cytol 4*:55–58, 1958.

18. RICHTER, G. W., and BESSIS, M. C. Commentary on hemosiderin. *Blood 25*:370–374, 1965.

19. RICHTER, G. W. The cellular transformation of injected colloidal iron complexes into ferritin and hemosiderin in experimental animals; a study with the aid of electron microscopy. *J Exp Med (Balt) 109*:197–216, 1959.

20. RICHTER, G. W. The nature of storage iron in idiopathic hemochromatosis and in hemosiderosis. Electron optical, chemical and serologic studies on isolated hemosiderin granules. *J Exp Med 112*:551–570, 1960.

21. LYNCH, K. M. (Symposium on occupational diseases of lungs; pathology of asbestosis. *A.M.A. Arch Industr Health 11*:185–188, 1955.

22. GLOYNE, S. R. The asbestosis body. *Lancet 1*:1351–1355, 1932.

23. KARRER, H. E. Electron microscopic study of the phagocytosis process in lung. *J Biophys Biochem Cytol 7*:357–366, 1960.

24. LOW, F. N., and SAMPAIO, M. M. The pulmonary alveolar epithelium as an entodermal derivative. *Anat Rec 127*:51–63, 1957.

25. TANAKA, H. "Electron Microscopic Studies on Vital Stain as Compared with Phagocytosis. A Concept of Segresomes." In *Electron Microscopy* (Vol. 2), BREESE, S. S., JR., Ed. Acad. Press, New York, 1962.

26. RATH, R. Form und Formänderung der Asbestosis-Körperchen. *Beitr Silikoseforsch 81*:1–10, 1964.

27. BESSIS, M. C., and BRETON-GORIUS, J. Iron metabolism in the bone marrow as seen by electron microscopy: a critical review. *Blood 19*:635–663, 1962.

28. MACNAB, G., and HARINGTON, J. S. Hemolytic activity of asbestos and other mineral dust. *Nature (London) 214*:522–523, 1967.

29. SUZUKI, Y., CHURG, J., and SMITH, W. Phagocytosis of asbestos fibers by epithelial cells. (abst.). *Lab Invest 18*:335, 1968.

30. HOLT, P. F., MILLS, J., and YOUNG, D. K. Experimental asbestosis with four types of fibers: Importance of small particles. *Ann NY Acad Sci 132*: 87–97, 1965.

We wish to thank Mr. Norman Katz, Norykazu Komatsu, Shigeo Kita, and Artie Prado for excellent technical assistance.

[*Illustrations follow*]

Legends to Figures

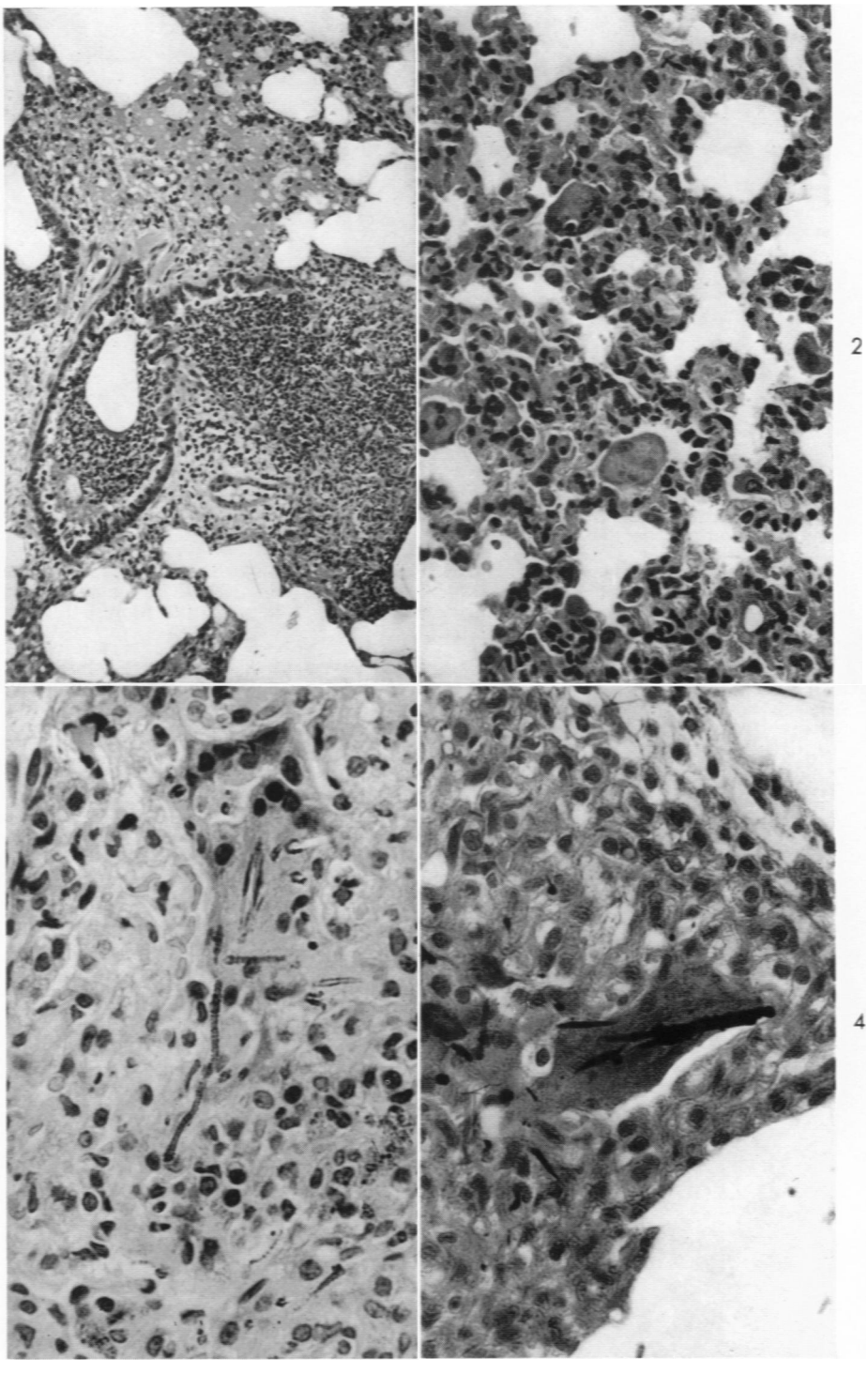
Time at the end of each legend represents elapsed time from the intratracheal instillation of asbestos. Fixation for electron microscopy is in osmium tetroxide, unless otherwise stated.

Fig. 1. Section of hamster lung. Acute inflammatory exudate fills a bronchiole and spills into peribronchiolar tissue. Many alveolar lumens are filled with edema fluid. One day. Hematoxylin and eosin. × 300.

Fig. 2. Alveolar walls are thick and very cellular. Mononuclear and multinucleated macrophages (giant cells) are seen in alveolar lumens. Both contain fine dust particles. No asbestos fibers or bodies can be identified by light microscopy. Sixteen days. Hematoxylin and eosin. × 700.

Fig. 3. There are many asbestos bodies in the shape of straight or bent, usually segmented, rods. Some lie in the cytoplasm of a giant cell. Alveolar architecture is almost completely lost. One year. Hematoxylin and eosin. × 1300.

Fig. 4. Same animal as in Fig. 3. Asbestos bodies stain strongly blue with Prussian blue techinque. A number of bodies are seen in cytoplasm of a giant macrophage. × 1100.



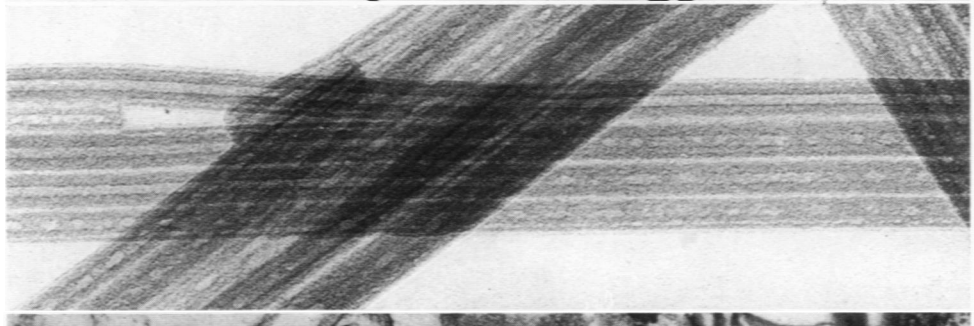
Figures 5–28 are electron micrographs.

Fig. 5. Soft chrysotile fibers from saline suspension. There is considerable variation in length and thickness. × 4000.

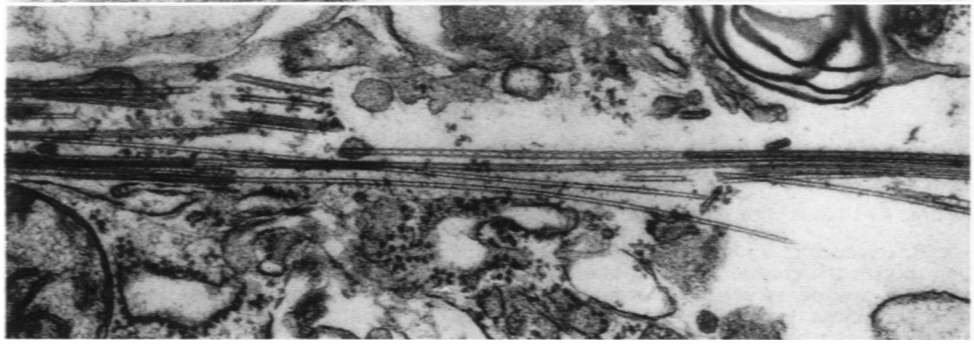
Fig. 6. Same preparation as in Fig. 5. Asbestos fibers consist of closely packed parallel arrays of unit fibers, the latter measuring about 300 Å in diameter. Unit fibers have a tubular capillary structure, with a central clear or interrupted (bridged) lumen surrounded by solid walls. × 225,000.

Fig. 7. Section of lung. Thin bundles and loose arrays of unit fibers are intermingled with cellular debris. Typical tubular structure of unit fibers is preserved. Note that these fibers remain naked or uncoated even after prolonged residence in the lung. Six months. × 44,000.

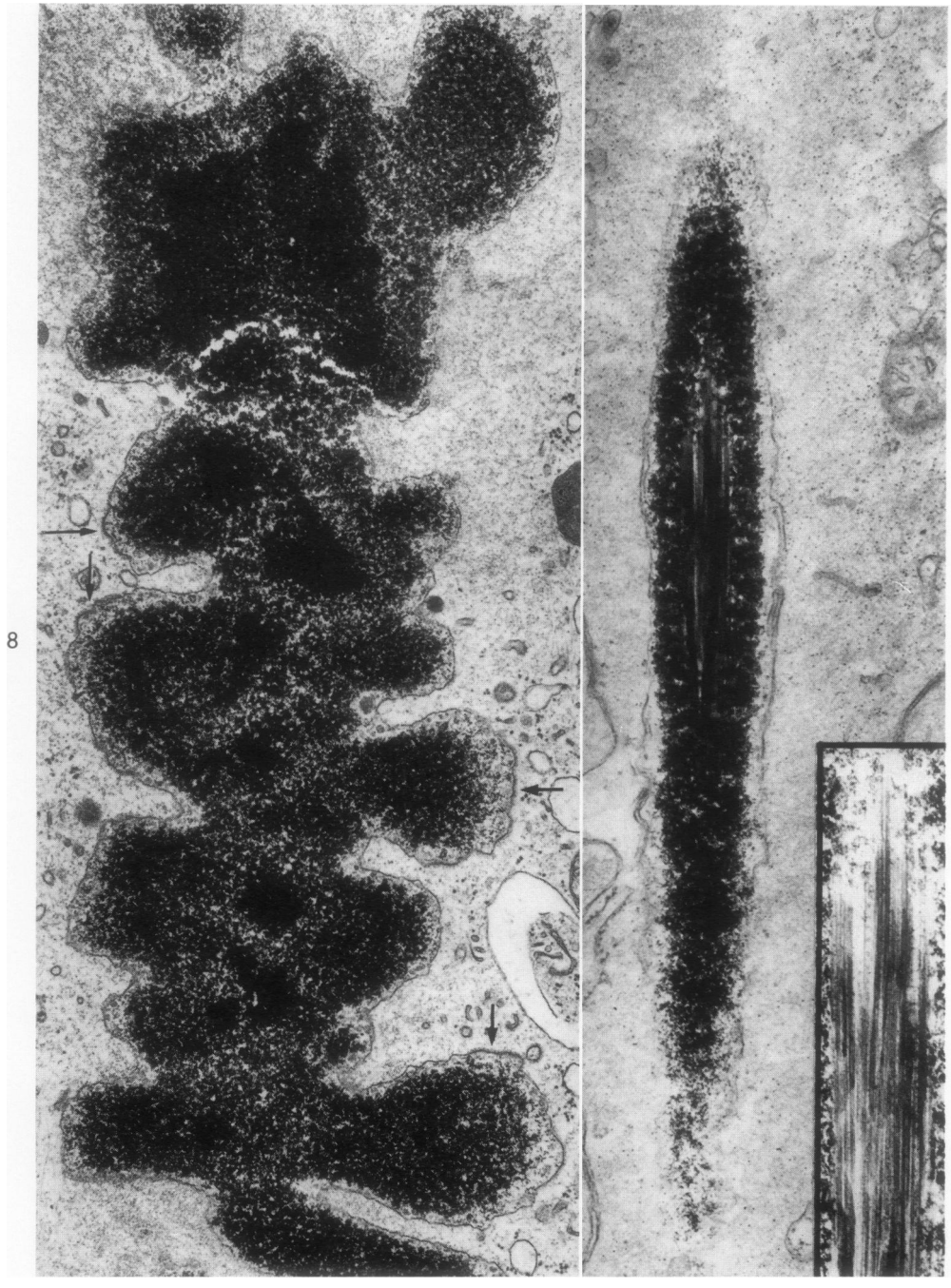
5



6



7



Figures 8–14 illustrate structure of the asbestos body.

Fig. 8. Intracytoplasmic segmented asbestos body in a longitudinal section. Plane of section misses the central asbestos fiber, but structure of the coat is well demonstrated. Iron micelles are densely packed toward the center, but are comparatively few along the periphery close to the limiting membrane. Fuzzy material *(arrows)* is attached to outer surface of membrane. Two years. Double fixation. × 30,000.

Fig. 9. Nonsegmented asbestos body showing central core of asbestos fibers. One year. × 33,000. **Inset.** Higher magnification of central core. Characteristic structure of chrysotile asbestos can be recognized. × 66,000.

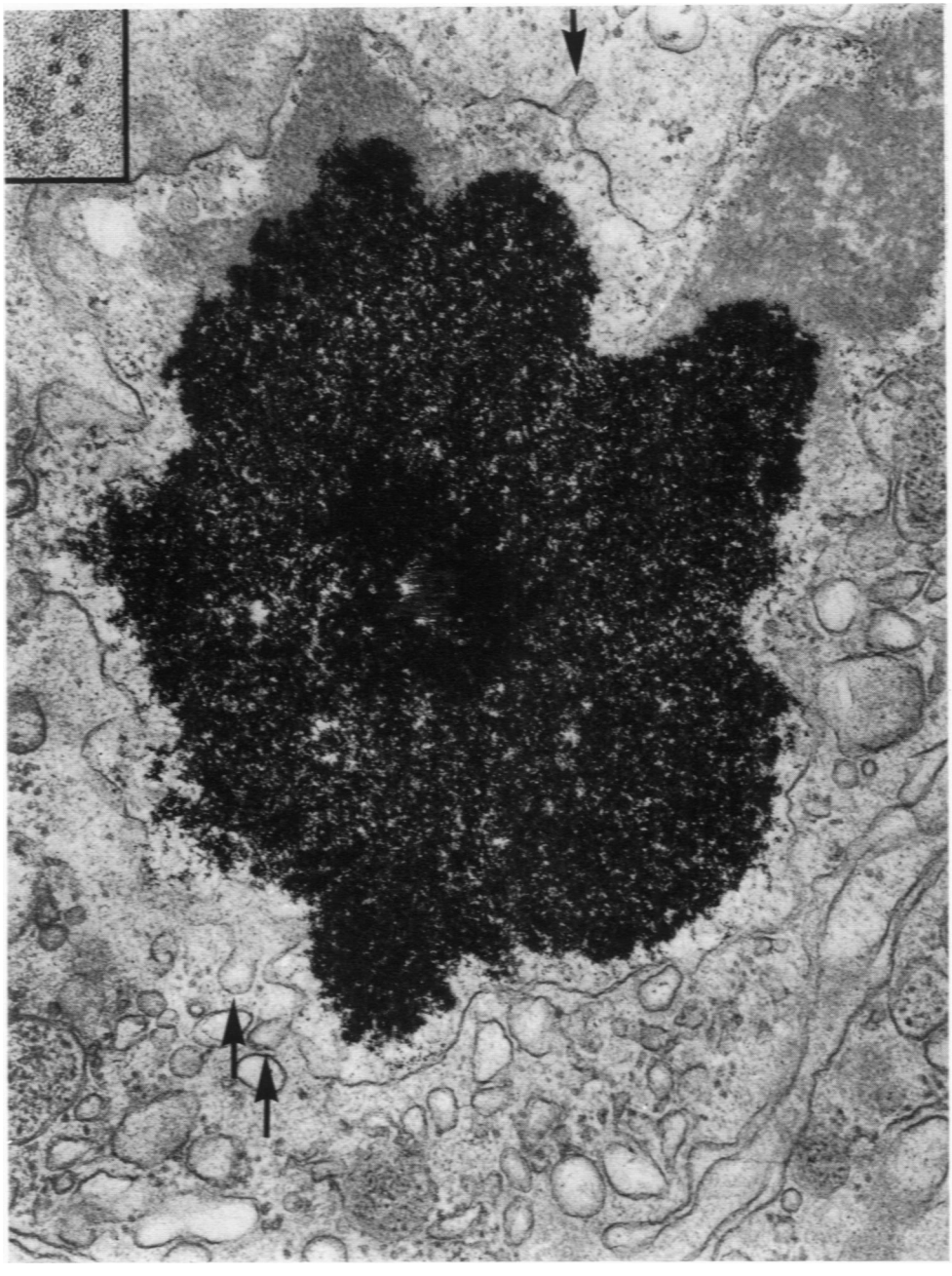
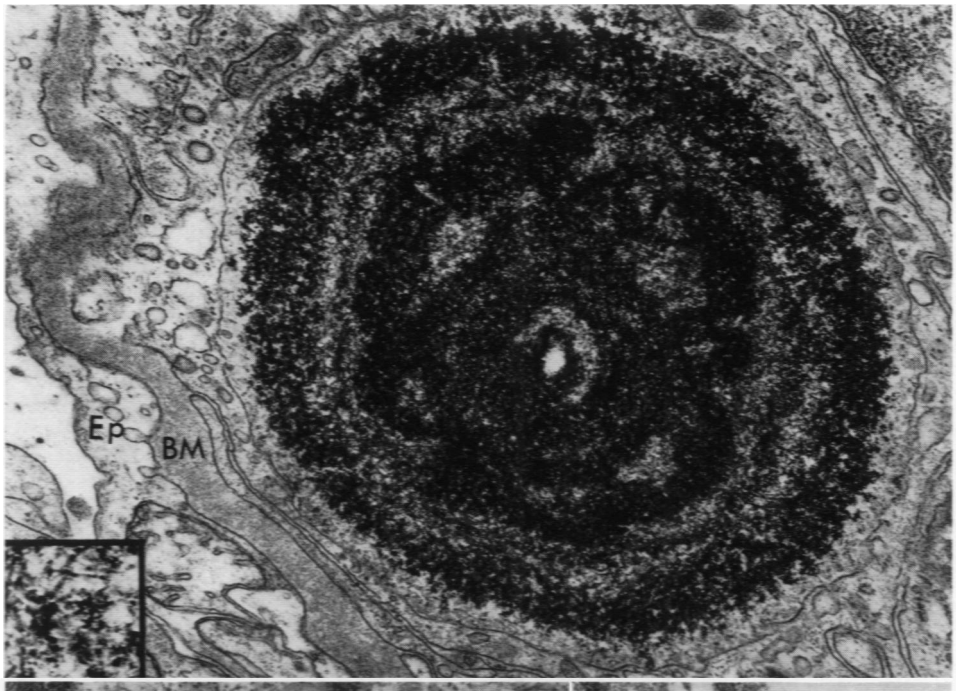


Fig. 10. Irregular asbestos body showing a wide space between compacted iron micelles and limiting membrane. This space contains gray amorphous material, best seen in upper part of figure. Arrows point to caveolae intracellulares. One year. × 48,000. **Inset.** High magnification of several iron micelles showing typical structure of ferritin. × 300,000.

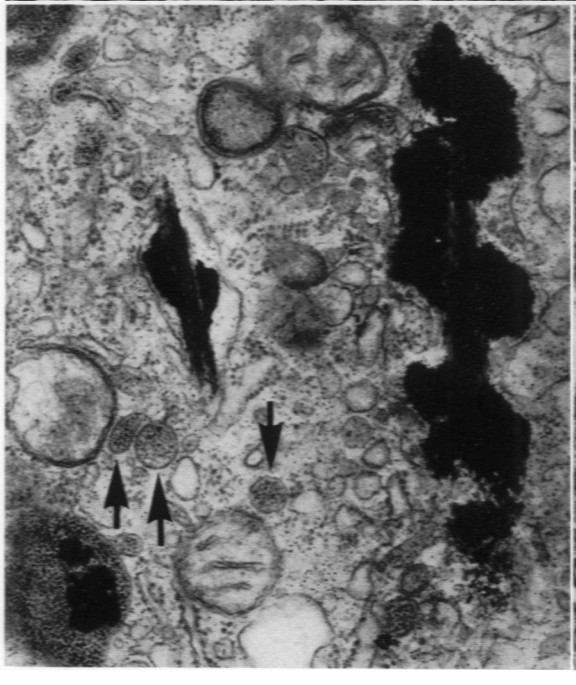
Fig. 11. Cross section of asbestos body showing irregular, layered structure. Cell containing the body is separated from alveolar epithelial cell *(Ep)* by a basement membrane *(BM)* and probably represents a derivative of alveolar septal cell. One year. × 6900. **Inset.** High magnification of light layer in the same body showing irregular filaments measuring about 30 Å in diameter. × 75,000.

Fig. 12. Two submicroscopic asbestos bodies, one measuring 0.5 μ and the other 1.3 μ in length. Despite the small size they are similar in structure to larger bodies. Note numerous vesicles *(arrows)* containing iron micelles in vicinity of bodies. One year. × 63,000.

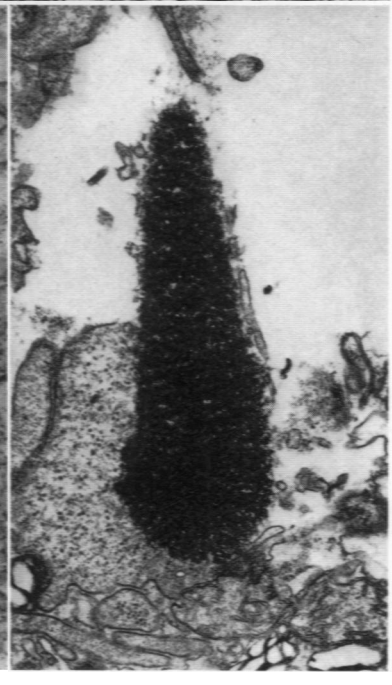
Fig. 13. A "free" body devoid of limiting membrane lies in an alveolar space. One year. × 14,000.



11



12



13

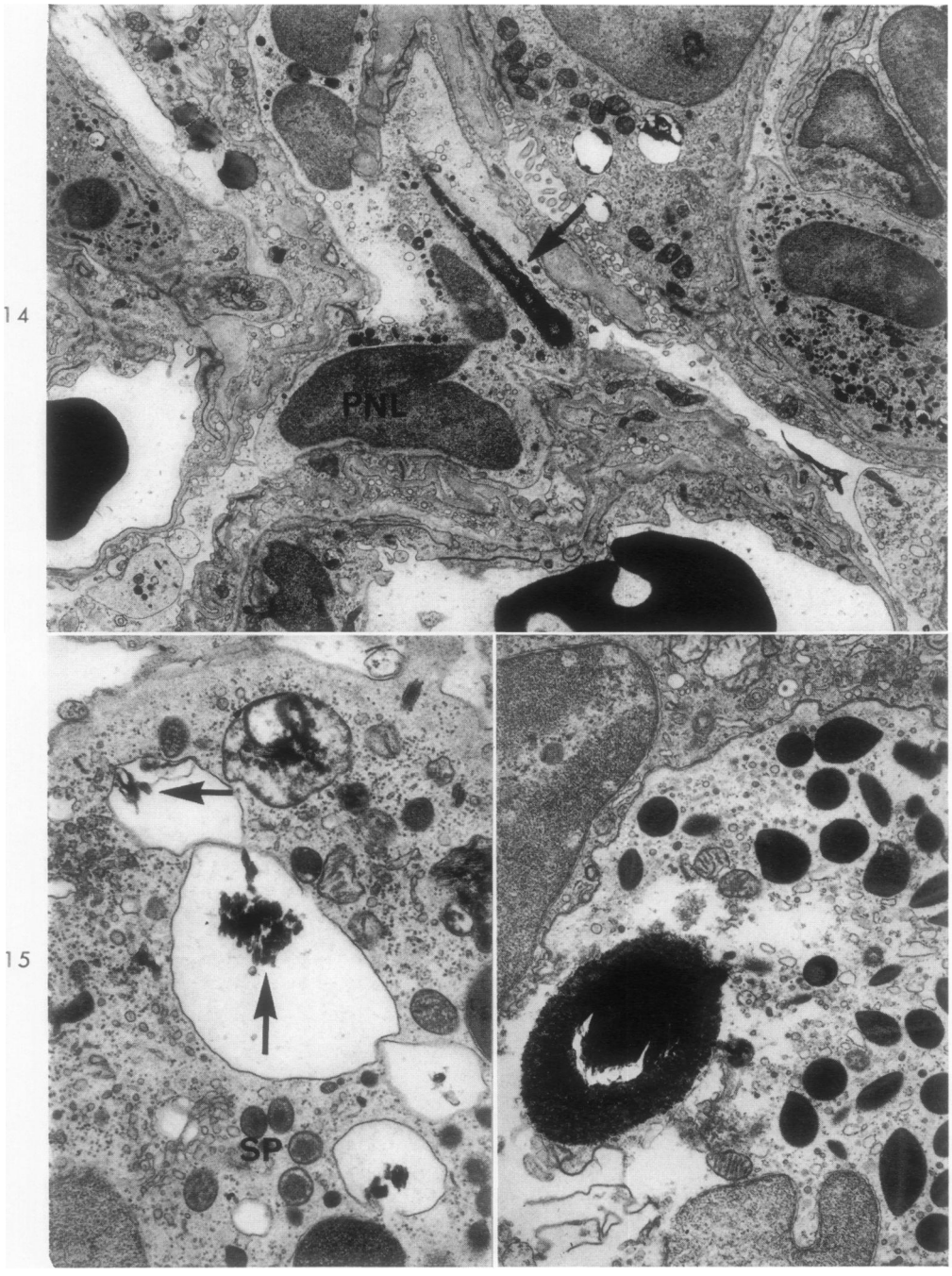
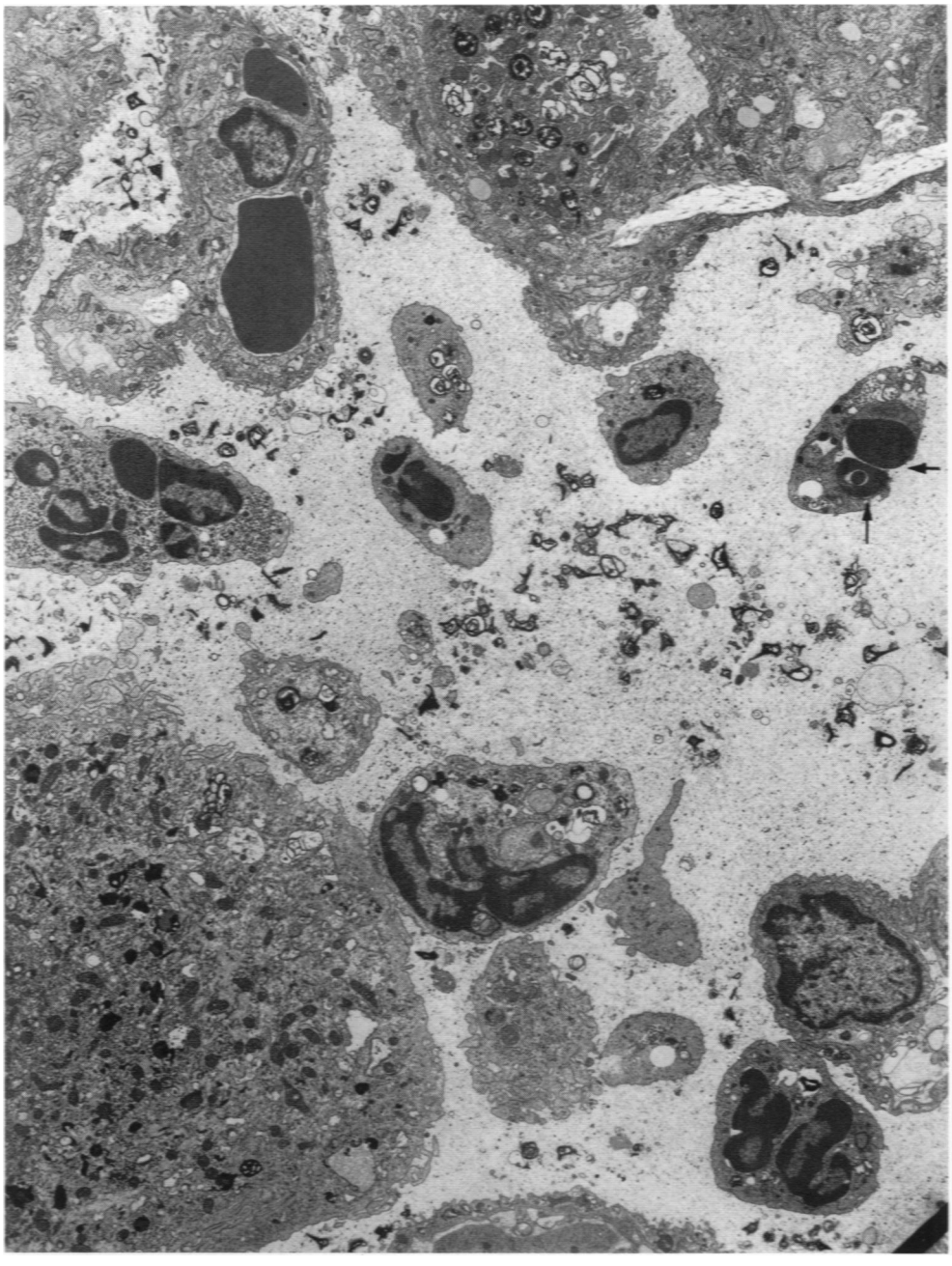


Fig. 14. Asbestos body *(arrow)* in cytoplasm of neutrophilic leukocyte *(PNL)*. One year × 9000.

Fig. 15. Asbestos fibers *(arrows)* in phagosomes of neutrophilic leukocyte. Specific (neutrophilic) granules *(SP)*. Seven days. × 17,000.

Fig. 16. Asbestos body in cytoplasm of eosinophilic leukocyte. Hemosiderin granules are absent. One year. × 11,000.

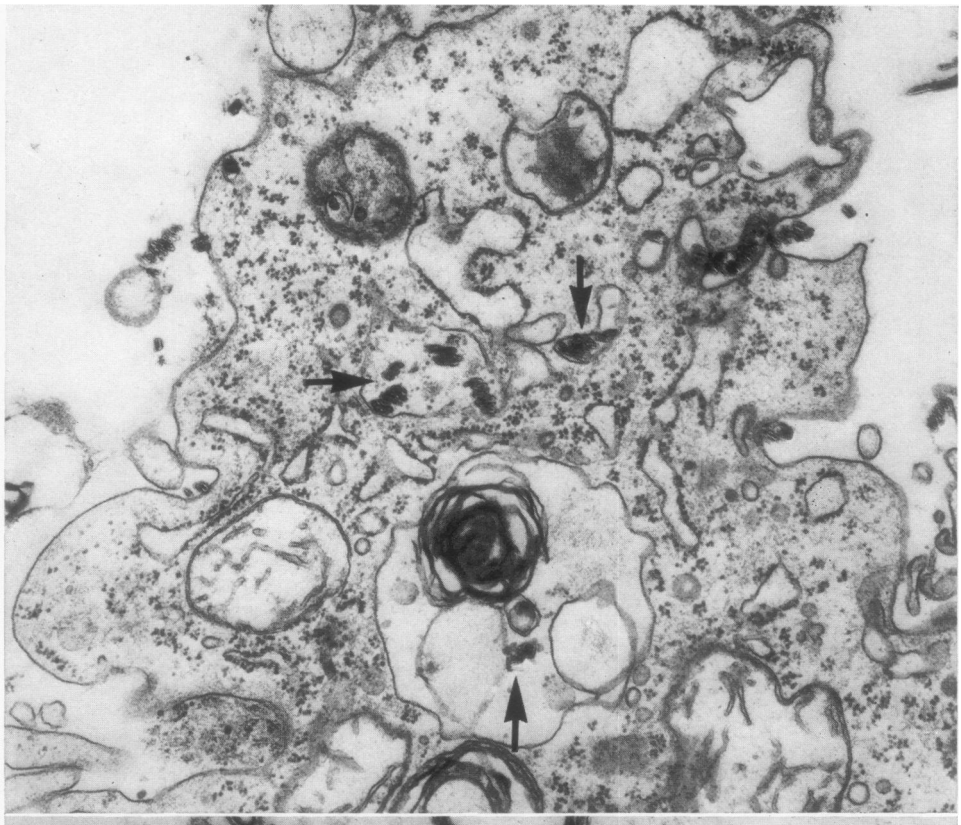


17

Figures 17–27 illustrate the development of the asbestos body.

Fig. 17. Alveolar lumen containing macrophages, polymorphonuclear leukocytes, and numerous osmiophilic lamellar bodies. Some macrophages contain ingested red blood cells *(arrows)*. Note that even at this magnification (× 4200) asbestos fibers are difficult to recognize. One day. Triple fixation.

18



19

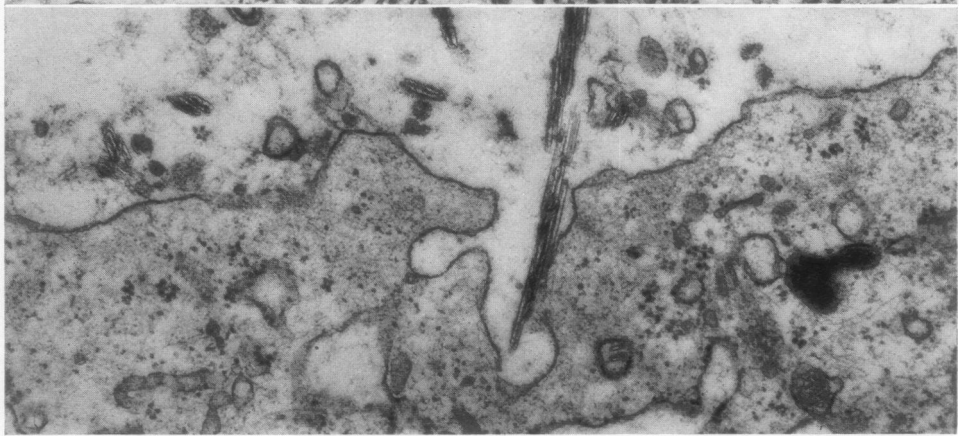


Fig. 18. Steps in phagocytosis of asbestos fibers by a macrophage. Some fibers are in the lumen, some are attached to the cell membrane, and others *(arrows)* are in the phagosomes. The fibers are less than 1 μ in length. One phagosome contains also an osmiophilic lamellar body. Three days. × 26,000.

Fig. 19. Phagocytosis of asbestos fibers. The fibers lie in a complex recess in direct contact with the plasma membrane. One day. Triple fixation. × 33,000.

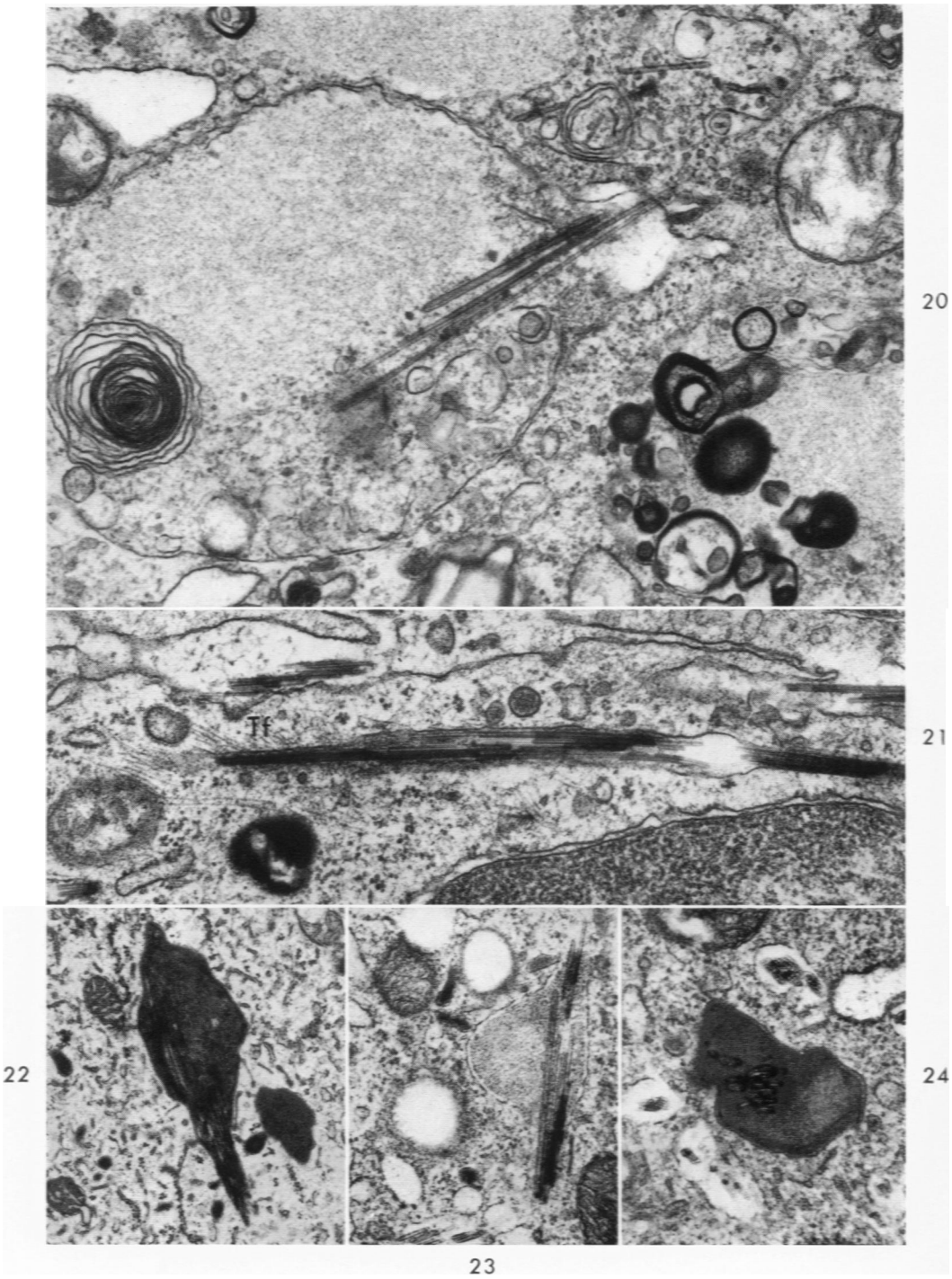


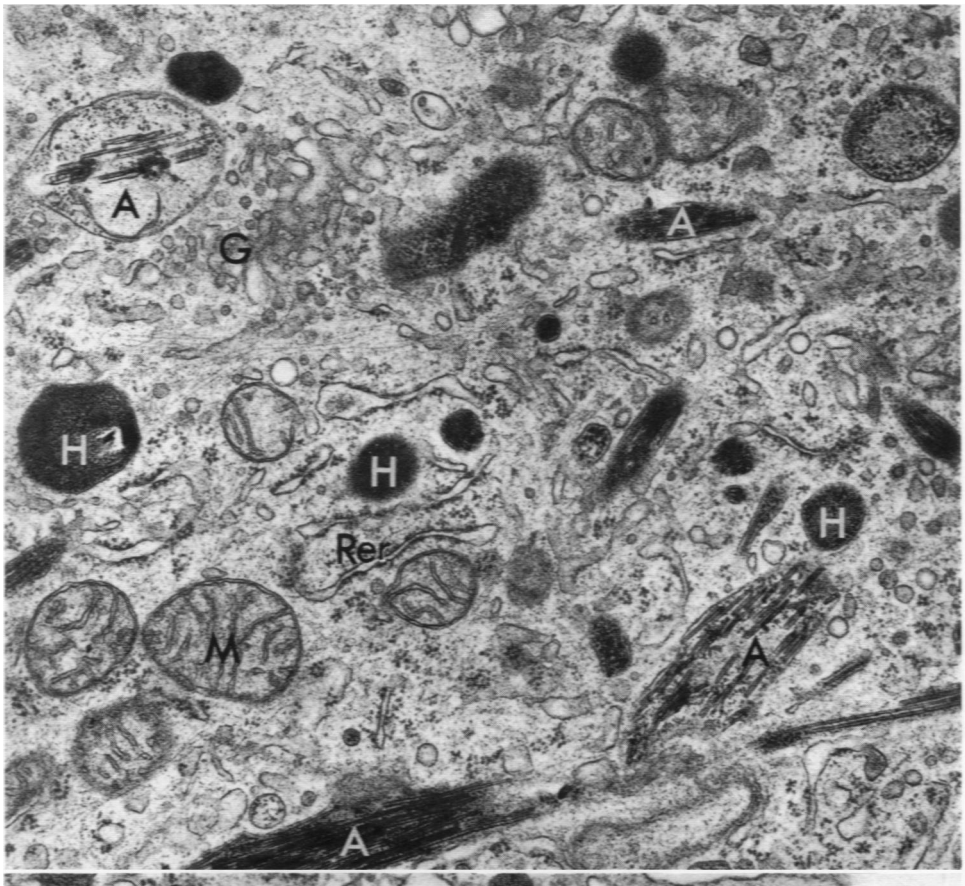
Fig. 20. A vacuole, apparently autophagic, is seen in cytoplasm of a macrophage. Vacuole contains asbestos fibers, an osmiophilic lamellar body, and cytoplasmic debris. Three days. Triple fixation. × 30,000.

Fig. 21. Markedly elongated phagosome is filled with asbestos fibers. Limiting membrane of phagosome is surrounded by well developed tonofilaments *(Tf)*. Seven days. Triple fixation. × 30,000.

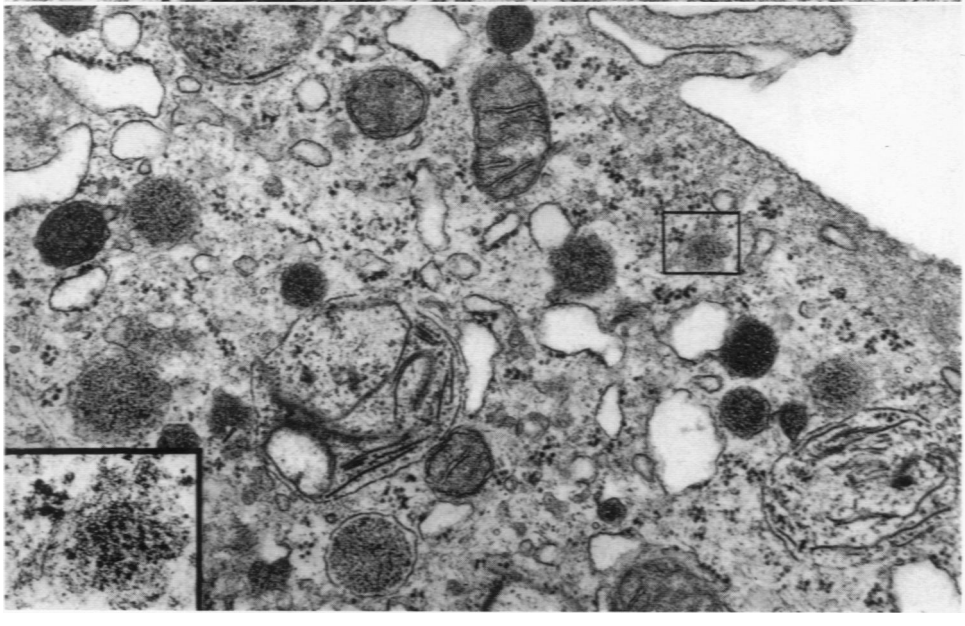
Fig. 22, 23, and 24. Phagosomes or lysosomes containing asbestos fibers. Note variability of shape and density. Five days. Triple fixation. Fig. 22, × 19,000; Fig. 23 × 26,000; Fig. 24, × 33,000.

Fig. 25. Cell organelles of an asbestos-containing macrophage. The Golgi area *(G)*, rough-surfaced endoplasmic reticulum *(Rer)*, and mitochondria *(M)* are well developed. In addition, there are many hemosiderin granules *(H)* and immature asbestos bodies *(A)*. Sixteen days. × 29,000.

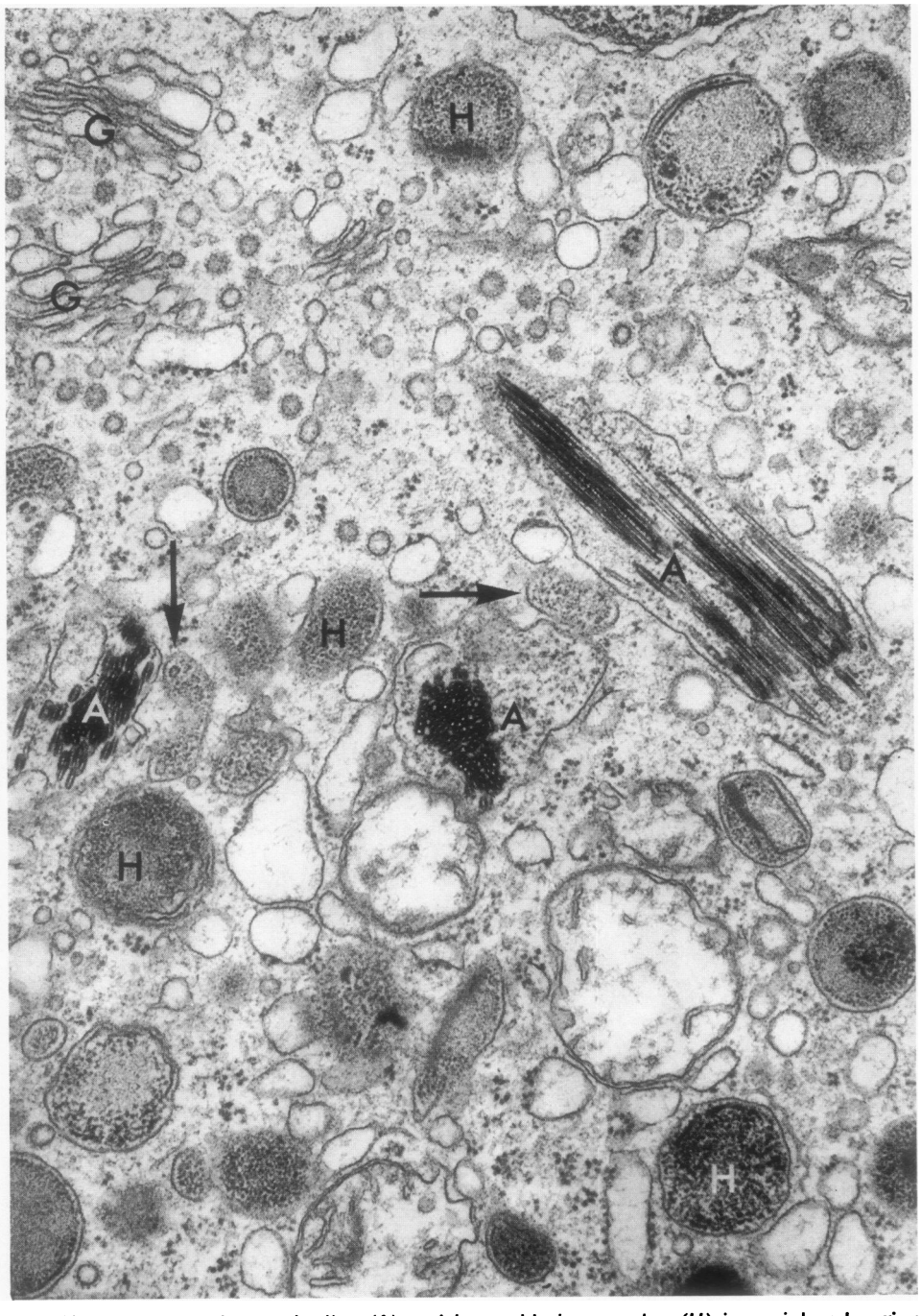
Fig. 26. Macrophage containing numerous hemosiderin granules, varying in size and in amount of iron. Double fixation. × 29,000. **Inset.** Higher magnification of area in box showing an aggregate of iron micelles, devoid of limiting membrane. × 74,000.



25

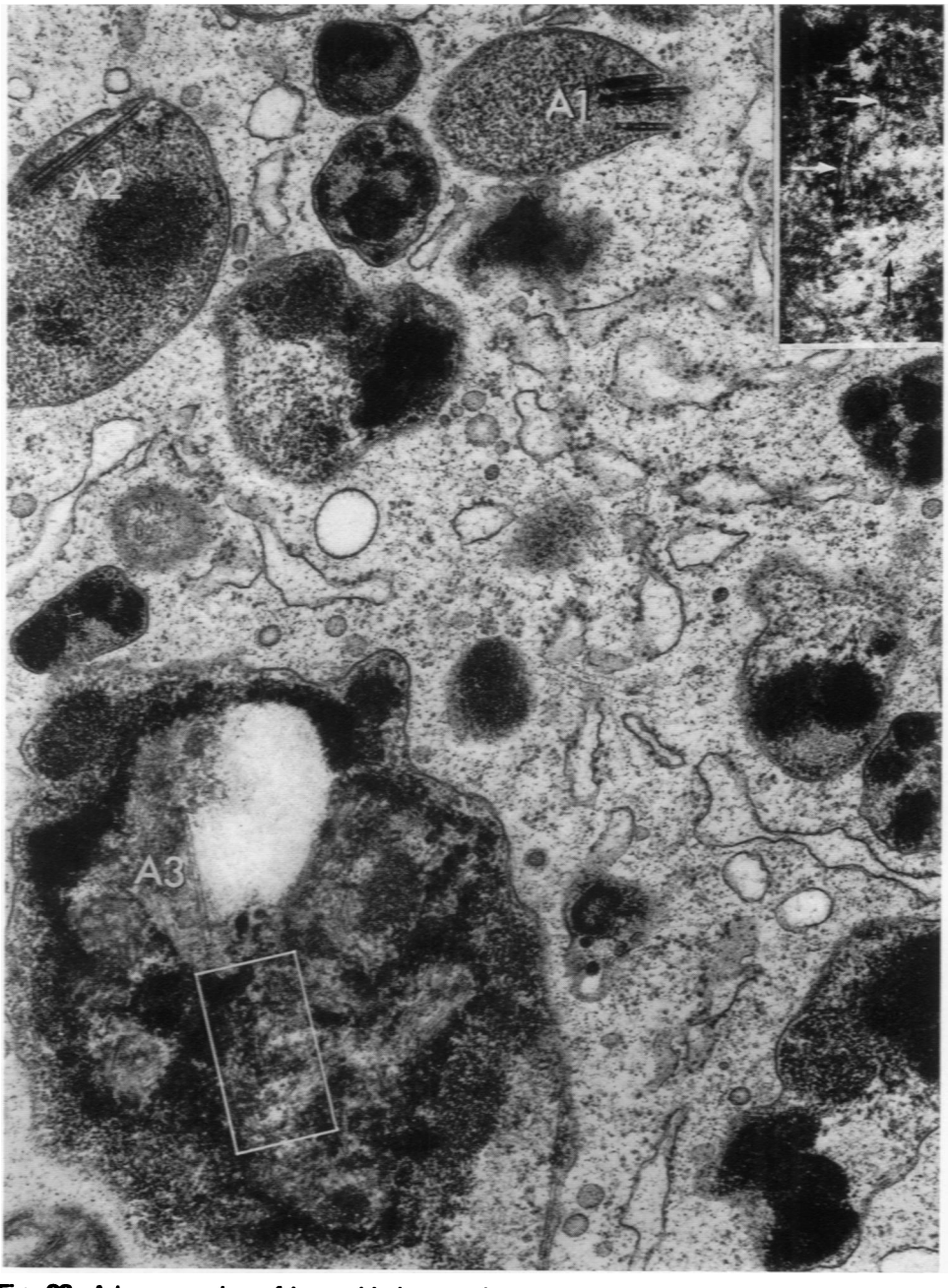


26



27

Fig. 27. Immature asbestos bodies *(A)* and hemosiderin granules *(H)* in peripheral region of a Golgi complex *(G)*. Some of the granules *(arrows)* are in close proximity to the bodies. The latter contain comparatively little iron. Six months. × 39,000.



28

Fig. 28. A large number of hemosiderin granules are seen in cytoplasm of alveolar macrophage. Some of the granules (*A1, A2, A3*) contain asbestos fibers. Note that the walls of unit fibers are very thin. One year. × 38,000. **Inset.** Higher magnification of rectangle in granule *A3*. There are several unit fibers with very thin walls. The appearance is suggestive of digestion or dissolution of fibers. × 68,000.